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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:

BLONDER et al.

Serial No.: 09/888,235

Filed: June 22, 2001

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For: "DELIVERY VEHICLE COMPOSITION AND METHODS FOR DELIVERING ANTIGENS AND OTHER DRUGS"

Group Art Unit 1648

Examiner: Li, Bao Q.

RULE 132 DECLARATION
OF CLAIRE M. COESHOTT
(37 C.F.R. § 1.132)

CERTIFICATE OF MAILING

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, DC 20231 ON April 22, 2003.

MARSH FISCHMANN & BREYFOGLE, LLP

BY: [signature]

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Madam:

Claire M. Coeshott, residing at 875 South Josephine Street, Denver, Colorado 80209 80027, declare as follows:

I am currently employed in the capacity of Director, Vaccine Technologies by RxKinetix, Inc., the assignee of the referenced U.S. Patent Application.

The attached Exhibit A is a summary of my technical qualifications.

The attached Exhibit B summarizes some tests (identified as Examples 11-16 for convenient reference) performed by me or by others at my direction concerning compositions for delivery of antigens. Examples 11-16 presented in Exhibit B concern formulation and testing of antigen delivery test compositions in which the antigen is formulated in an aqueous liquid with an adjuvant material and a polymer of a type and in an amount to impart reverse-thermal viscosity behavior to the composition. Antigens subject to the testing include tetanus toxoid (TT), diphtheria toxoid (DT) and recombinant anthrax protective antigen (rPA); adjuvant materials tested include those containing chitosan or CpG dinucleotide motifs (CpG); and the polymer for all tests is Pluronic® F127 polymer. Studies in mice compared the performance of these test compositions as compared to comparison compositions in which the antigen is differently formulated. Results of these mice studies are

discussed in Exhibit B, with tabular results of mice antibody response data for Examples 12-15 being presented in attached Exhibits C-F. The results of the mice studies presented in Exhibits B-F demonstrate a high level of antibody response to the test composition, and with the antibody response to the test composition most often being both faster to develop and attaining a higher level than the antibody response to the comparison compositions, as indicated by antibody assays. The attainment of a higher level of antibody response is obviously important. Perhaps more important, however, is the faster antibody response to the test compositions. In a high-risk situation, such as an epidemic, development of quicker immunization response following antigen administration may mean the difference between someone surviving or not surviving the situation. This more rapid response to immunization is surprising as it might be expected that administering the antigen in the reverse-thermal viscosity composition would delay distribution of the antigen to the relevant cells of the immune system, thus slowing any immune response.

Example 11 presents a general procedure for preparing formulations and for performing and obtaining antibody assays to determine antibody response. Examples 12-16 discuss preparation of specific formulations and mice studies on those particular formulations, generally as described in Example 11 except as noted.

In Example 12, test compositions with TT are formulated with 16.25% (w/w) Pluronic®F127 polymer and with varying amounts of an adjuvant material containing chitosan (0.5, 0.17, or 0.05% (w/w) of the adjuvant material). Comparative compositions with TT are also formulated with only the adjuvant material or with only the polymer. The test compositions demonstrate higher IgG antibody response at both two weeks and at five weeks following a single subcutaneous administration of 0.5 Lf TT than the comparable comparative compositions, as clearly summarized in the following table, which provides data for geometric mean and average IgG antibody titers in serum samples from the mice studies on the different compositions.

Chitosan Adjuvant Material Content	IgG Antibody Titers – Geometric Mean and (Average)		
	Test Comp. With Both Adj. Mtl. & Polymer	Comparative Comp. With Only Adj. Mtl.	Comparative Comp. With Only Polymer
Two Weeks Following Administration			
0.5% (w/w)	413 (445)	162 (396)	
0.17% (w/w)	497 (665)	337 (467)	
0.05% (w/w)	252 (271)	215 (236)	
0% (w/w)			27 (56)
Five Weeks Following Administration			
0.5% (w/w)	14,132 (18,836)	4748 (5403)	
0.17% (w/w)	11,201 (13,194)	9,119 (11,442)	
0.05% (w/w)	5,437 (7,055)	4,862 (6,165	
0% (w/w)			122 (289)

As summarized in the above table, the comparative composition formulated with only Pluronic® F127 polymer, and no adjuvant material, performed poorly. Comparative compositions formulated with only the adjuvant material, and no Pluronic® F127 polymer performed better than comparative compositions formulated with only Pluronic® F127 polymer, but the test compositions, formulated with both the adjuvant material and the Pluronic® F127 polymer, performed the best.

In Example 13, test compositions with TT are formulated with 16.25% (w/w) Pluronic® F127 polymer and with 20% (v/w) adjuvant material containing CpG. Comparative compositions with TT are also formulated without the Pluronic® F127 polymer, but with the CpG-containing adjuvant material with and without the addition also of glycerol or incomplete Freund's adjuvant (IFA). The test compositions demonstrate higher IgG antibody response following a single subcutaneous administration of 0.5 Lf TT than the comparative compositions. It is of particular interest to point out that IFA is considered a "gold standard" for adjuvants used in immunization of experimental animals and that the test composition is an improvement. In Example 14, test compositions with TT are formulated with 16.25% (w/w) Pluronic® F127 polymer and with various amounts of an adjuvant material containing CpG (20, 6.7 or 2 % (v/w) of the adjuvant material). Comparative

compositions with TT are also formulated with only the adjuvant material or with only the polymer. The test compositions consistently demonstrate higher IgG antibody response at two, four and eight weeks following a single subcutaneous administration of 0.5 Lf TT than the comparable comparative compositions, as clearly summarized in the following table, which provides data for geometric mean and average IgG antibody titers in serum samples from the mice studies on the different compositions.

CpG Adjuvant Material Content	IgG Antibody Titers – Geometric Mean and (Average)		
	Test Comp. With Both Adj. Mtl. & Polymer	Comparative Comp. With Only Adj. Mtl.	Comparative Comp. With Only Polymer
Two Weeks Following Administration			
20%(v/w)	6,974 (7,705)	5,287 (5,792)	
6.7% (v/w)	1,761 (1,969)	476 (554)	
2% (v/w)	694 (792)	264 (284)	
0% (vw)			623 (780)
Four Weeks Following Administration			
20%(v/w)	14,768 (32,636)	6,050 (8,309)	
6.7% (v/w)	77,632 (101,667)	3,225 (3,472)	
2% (v/w)	14,037 (18,054)	2,243 (2,282)	
0% (v/w)			626 (884)
Eight Weeks Following Administration			
20%(v/w)	39,903(77,778)	14,429(46,467)	
6.7% (v/w)	76,792(172,083)	8,566(11,619)	
2% (v/w)	17,065(27,739)	4,034(4,714)	
0% (v/w)			345(926)

As summarized in the above table, the comparative composition formulated with only Pluronic® F127 polymer, and no adjuvant material, performed poorly. Comparative compositions formulated with only the adjuvant material, and no Pluronic® F127 polymer performed better than comparative compositions formulated with only Pluronic® F127 polymer, but the test compositions, formulated with both the adjuvant material and the Pluronic®F127 polymer, consistently performed the best. The

values for the 20% test composition may be lower than expected in this example due to technical difficulties performing the assay.

In Example 15, test compositions with DT are formulated with 16.25% (w/w) Pluronic®F127 polymer and with 20% (v/w) adjuvant material containing CpG. Comparative compositions with DT are also formulated without the Pluronic® F127 polymer, but with the adjuvant material containing CpG. The test compositions demonstrate attainment of a higher IgG antibody response following a single subcutaneous administration of 1 Lf DT than the comparative compositions, although at later times (after 12 weeks) following administration, the comparative compositions do result in similiar IgG antibody responses.

In Example 16, test compositions with rPA are formulated with 16.25% (w/w) Pluronic® F127 polymer and with 20% (v/w) adjuvant material containing CpG. Comparative compositions with rPA are also formulated without the Pluronic® F127 polymer, but with either the CpG-containing adjuvant material or alternatively with aluminum hydroxide (alum). The test compositions demonstrate attainment of a higher IgG antibody response following a single subcutaneous administration of 25 μg rPA than the comparative compositions including the alum. Also, the test compositions resulted in significantly higher toxin neutralization antibody titers than either the comparison compositions with the CpG-containing adjuvant or the comparison compositions containing alum. The toxin neutralization assay is a measure of the ability of the test composition to raise an antibody response that protects cells against challenge with anthrax lethal toxin and therefore is an excellent indicator of the effectiveness of the test composition.

All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. §1001) and may jeopardize the validity of this patent application or any patent issuing thereon.

Respectfully submitted,

Date: 4/22/03

By: C M Coeshott

Claire M. Coeshott

EXHIBIT A
TO RULE 132 DECLARATION OF
CLAIRE M. COESHOTT

BIOGRAPHICAL SKETCH AND TECHNICAL QUALIFICATIONS

NAME Coeshott, Claire M.	TITLE Director, Vaccine Technologies

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
University of Bristol, England	B.Sc., 1st class, honors	1978	Pathology
University of Bristol, England	Ph.D.	1982	Immunology

RESEARCH AND PROFESSIONAL EXPERIENCE:

Employment

1981-1982	Research Assistant, Department of Pathology, University of Bristol, England.
1982-1985	Research Fellow, National Jewish Hospital and Research Center, Denver, Colorado.
1985-1988	Research Associate, Division of Membrane Biology, Medical Biology Institute, La Jolla, California.
1988-1994	Group Leader: Immunology, Cortech Inc., Denver, Colorado.
1991-1994	Team Leader: Lupus Project, Cortech Inc., Denver, Colorado.
1994-1996	Group Leader: Immunoassay Methods, Biopharmaceutics Department, Cortech Inc., Denver, Colorado.
1996-1997	Group Leader: Protease Inhibitor Program, Pharmacology Department, Cortech Inc., Denver, Colorado.
1997-1998	Research Fellow and Group Leader, Biology Department, Ribozyme Pharmaceuticals Inc., Boulder, Colorado.
1999-2000	Senior Scientist, Ceres Pharmaceuticals Ltd., Denver, Colorado.
2000-2002	Senior Scientist, RxKinetix Inc. Louisville, Colorado.
2002	Director, Vaccine Technologies, RxKinetix Inc. Louisville, Colorado.

Synopsis Of Industrial Experience

RxKinetix Inc. 2000 – present
Project leader for vaccine program to evaluate proprietary formulations for vaccine delivery. Coordinate research effort in house and with outside collaborators. Develop

assays for measurement of antibody and T cell responses to formulations. Liaise with business development and legal departments for optimal positioning of technology.

Globeimmune, Inc (formerly Ceres Pharmaceuticals Ltd.) 1999 – 2000

Employed as both bench scientist and manager for a SBIR-funded project to develop a genetically-engineered microorganism as an HIV vaccine. Designed and executed *in vivo* and *in vitro* experiments for vaccine program:

- obtained Proof of Principle for vaccine candidate using a tumor protection model in mice.

Ribozyme Pharmaceuticals Inc. 1997 – 1998

Led multidisciplinary project to develop ribozyme-based therapeutic to treat chemoresistance in cancer. Team consisted of 3 Ph.Ds. and 4 RAs. In addition, was line manager for 3 RAs within Biology Group:

- co-ordinated synthesis of ribozymes, designed in vitro experiments with RNA endpoints (RNase protection assay and Taqman analysis) and phenotypic endpoints (apoptosis).
- designed and oversaw in vivo experiments to test lead compounds using human cancer cell line xenografts in athymic mice

Cortech Inc. 1989 – 1997

Immunology Program: Basic Research

Developed Immunology program using multivalent arrays of haptens on large molecular weight carriers such as dextran to suppress or stimulate hapten-specific antibody responses in mice. Outcomes of program:

- patent issued (November 1996) addressing stimulatory aspects of technology which formed basis of vaccine program at Cortech.
- filing of an IND application (March 1995) and subsequent completion of phase I clinical trial for a specific immunomodulator, CI-0694, to suppress sulfamethoxazole hypersensitivity in AIDS patients .

Set up tissue culture laboratory as service facility for providing monoclonal antibodies to other projects:

- developed and characterized peptide-specific helper T cell hybridomas and their responses to various Cortech compounds.
- demonstrated activity of Cortech compounds for cytotoxic T cell induction.
- developed and characterized monoclonal antibodies against fibrinopeptides and bradykinin antagonists.

Protease Inhibitor Program: Research

Program addressed potential of novel synthetic, substrate-based compounds to inhibit enzymatic degradation of tissues and release of various cytokines:

- designed and executed assays for measuring impact of inhibitors on cytokine production (TNFα, IL-1β, IL-2, IL-8) from whole blood as well as from various cell types including THP-1 monocytic cell line, Jurkat, neutrophils and monocytes isolated from human peripheral blood.
- oversaw development of extracellular matrix assay to test inhibition of radiolabelled matrix degradation.

Managerial

As leader of Lupus project, coordinated a team of up to three Ph.Ds. and four RAs in the production of compound to suppress nephritis occurring in the autoimmune disease, systemic lupus erythematosus:

- initiated and oversaw collaborations with researchers in field to assess recognition of Cortech compounds by antibodies from human SLE patients.
- developed ELISPOT assay to measure anti-DNA and anti-histone antibody-secreting cells.
- designed and executed all in vivo experiments to monitor the effects of these constructs in lupus-prone mice.
- lead compound identified.

Pre-clinical Research

As member of Biopharmaceutics department, supervised two senior- level RAs and one post-doctoral researcher:

- developed immunoassays to measure specific antibody responses in AIDS patients entering phase I clinical trial of CI-0694. ELISA and competition ELISA for IgM, IgA and IgG developed and subsequently used for measurement of antibodies in samples from phase I trial. Liased with AIDS Clinical Trial Group (ACTG) in evaluation of CI-0694.
- collaborated with physicians at Denver General Hospital in study to investigate correlation between antibody levels and failure of desensitization to sulfamethoxazole.
- coordinated clinical studies to examine efficacy of elastase inhibitor, CE-1037, in cystic fibrosis and ARDS: defined sample handling procedures for BALF and sputum; participated in site visits and initiation of two clinical trials.
- wrote research reports and SOPs; reviewed INDs, clinical protocols and other documents.

Awards, Honors, Grants

1. Leukemia Society of America Special Fellowship, July 1987 - July 1990.
2. University of Bristol Postgraduate Scholarship, 1978 - 1981.

Memberships

British Society for Immunology

Patents

1 issued; 2 applications

Selected Publications

Grace S.A., Elson, C.J. and Coeshott, C.M. Production of anti-host IgG by transfer of primed histocompatible cells. **Clin. Exp. Immunol. 39:449, 1980.**

Elson, C.J. and Coeshott, C.M. Tolerance of allotypic determinants induced by lymphoid cells from congenic mice bearing the allotype. **Immunol. 43:281, 1981.**

Coeshott, C.M. and Grey, H.M. Transfer of antigen presenting capacity to la negative cells upon fusion with la-bearing liposomes. **J. Immunol. 134:1343, 1985.**
Gay, D., Coeshott, C.M., Golde, W., Kappler, J. and Marrack, P. The Major Histocompatibility Complex-restricted antigen receptor on T cells IX. Role of accessory molecules in recognition of antigen plus isolated IA. **J. Immunol. 136:2026, 1986.**
Coeshott, C.M., Chesnut, R.W., Kubo, R.T., Grammer, S.F., Jenis, D.M. and Grey, H.M. Ia-specific mixed leukocyte reactive T cell hybridomas: Analysis of their specificity by using purified class II MHC molecules in a synthetic membrane system. **J. Immunol. 136:2832, 1986.**
Blodgett, J.K., Coeshott, C.M., Roper, E.F., Ohnemus, C., Allen, L.G., Kotzin, B.L. and Cheronis, J.C. Synthesis and characterization of novel antigen-specific immunosuppressive agents and their utilization in the (NZB x NZW)F1 murine model of systemic lupus erythematosus. **Proc. Amer. Pep. Symp. 12: 873, 1992.**
Coeshott, C., Allen, L., McLeod, D., Cheronis, J. and Kotzin, B. Antigen-specific suppression of antibody responses: implications for vaccine design. **Vaccines 95. Cold Spring Harbor Laboratory Press, 1995.**
De la Cruz, V.F., Cook, C., Allen, L., Strong, P., Blodgett, J., Ohnemus, C., McCall, C., Goodfellow, V., McLeod, D., Gross, K., Cheronis, J. and Coeshott, C. Antigen-specific Immunomodulation (ASIM): the rational design of molecules that are inherently immunogenic. **Vaccines 95. Cold Spring Harbor Laboratory Press, 1995.**
Pilyavskaya, A., Wieczorek, M., Asztalos, J., Coeshott, C., Francis, M.D. and Blodgett, J. Purification of $F(ab')_2$ and Fab' fragments from the T cell receptor-specific monoclonal antibodies, F23.1 and KJ16, and preparation of conjugates with dexamine. **J. International Bio-chromatography, 3: 215, 1996.**
Coeshott, C., Ohnemus, C., Pilyavskaya, A., Ross, S.E., Wieczorek, M. Kroona, H., Leimer, A. and Cheronis, J. Converting enzyme-independent release of TNFα and IL-1β from stimulated THP-1, a human monocytic cell line, in the presence of activated neutrophils or purified proteinase 3. **Proc. Natl. Acad. Sci. USA, 96: 6261, 1999.**
Stubbs, A.C., Martin, K.S., Coeshott, C., Skaates, S.V., Kuritzkes, D.R., Bellgrau, D., Franzusoff, A., Duke, R.C. and Wilson, C.C. Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. **Nature Medicine 7:625-629, 2001.**
Westerink, M.A.J., Smithson, S.L., Srivastava, N., Blonder, J., Coeshott, C., and Rosenthal, G.J. Projuvant™ (Pluronic F127®/chitosan) enhances the immune response to intranasally administered tetanus toxoid. **Vaccine 20: 711-723, 2001.**

EXHIBIT B
TO RULE 132 DECLARATION OF
CLAIRE M. COESHOTT

EXAMPLE 11: General procedure for preparing and testing antigen delivery compositions

Preparation of formulations: Pluronic® F127 polymer (National Formulary pharmaceutical grade, BASF, Washington, NJ) stock solution was prepared at 34% (w/w) by dissolving in ice-cold PBS with complete dissolution achieved by storing overnight (ON) at 4°C. Protasan® (Chitosan chloride, ultrapure CL 213; Pronova Biomedical, Oslo, Norway; MW = 272,000; 84% deacetylated) stock solutions were prepared at 3% (w/w) in 1.0 % (v/v) acetic acid in sterile water (USP grade) and were heated at 37°C to dissolve. An adjuvant containing CpG dinucleotide motifs (CpG) was obtained from Qiagen (ImmunEasy™, proprietary formulation containing CpG of Qiagen Inc. Valencia, CA) and was added to formulations according to the manufacturer's instructions. The antigens evaluated include recombinant anthrax protective antigen (rPA), tetanus toxoid (TT), and diphtheria toxoid (DT). Adjuvants, such as those containing chitosan or CpG, were also added to the formulations. Unless otherwise noted, the stock solutions were mixed together to prepare formulations containing various combinations of antigen, adjuvant and Pluronic® F127 polymer.

Immunization studies in mice: Balb/c female mice (Harlan, Indianapolis, IN) 6 to 8 weeks of age were used for these studies. Groups of mice were immunized once subcutaneously (s.c.) with antigens in various formulations on day 0.

Antibody assays: The serum antibody responses to antigens were measured by ELISA. Wells of 96 well Nunc Maxisorb microtiter plates (Nunc, Gaithersburg, MD) were coated with the appropriate concentration of antigen in PBS. Plates were washed with PBS/0.05% Tween 20 and blocked with 1% bovine serum albumin (BSA) (Fisher Scientific, Pittsburgh, PA). Serum samples were serially diluted in PBS/0.1% BSA/0.05% Tween 20 (PBST) and added to wells in triplicate. Following incubation, plates were washed and goat anti-mouse IgG γ chain specific horseradish peroxidase (HRP)-labeled conjugate (Southern Biotechnology Associates Inc., Birmingham, AL) was added in PBST. After further incubation, antibody binding was detected with substrate buffer containing tetramethylbenzidine (TMB) (Sigma-Aldrich). Absorbance was read with an EIA reader (Molecular Devices, Sunnyvale, CA). Antibody titer was defined as the reciprocal of the dilution of serum that would yield an optical density of 0.5.

Statistics: Data were analyzed for differences using Students t test. A probability (p) of 0.05 or less was considered significant. Outliers were identified by Grubb's test.

Testing of performance of specific formulations with antigens TT, DT and rPA are discussed below in Examples 12-16.

EXAMPLE 12: TT with chitosan-containing adjuvant in the composition

Preparation of formulations: TT and Pluronic® F127 stock solutions were prepared as described in Example 1. Protasan® stock solution was prepared at 3% (w/w) in 1.0 % (v/v) acetic acid in sterile water (USP grade). Tetanus toxoid (Accurate Chemical & Scientific, Westbury, NY)

contained 1058 Lf/ml and 2204 Lf/mg protein nitrogen. The various stock solutions were mixed together to form vaccine compositions for testing, as follows:

(i) TT (5 Lf/ml), 0.5% (w/w) chitosan and 16.25% (w/w) Pluronic® F127;
(ii) TT (5 Lf/ml), 0.17% (w/w) chitosan and 16.25% (w/w) Pluronic® F127;
(iii) TT (5 Lf/ml), 0.05% (w/w) chitosan and 16.25% (w/w) Pluronic® F127;
(iv) TT (5 Lf/ml) and 0.5% (w/w) chitosan (no Pluronic® F127);
(v) TT (5 Lf/ml) and 0.17% (w/w) chitosan (no Pluronic® F127);
(vi) TT (5 Lf/ml) and 0.05% (w/w) chitosan (no Pluronic® F127); and
(vii) TT (5 Lf/ml) and 16.25% (w/w) Pluronic® F127 (no chitosan).

Immunization studies in mice: Balb/c female mice (Harlan), 6 to 8 weeks of age, were used for these studies. Mice were immunized once s.c with 0.5 Lf TT in the various formulations on day 0.

Antibody assays: The serum antibody responses to TT were measured by ELISA. Wells of 96 well Nunc Maxisorb microtiter plates (Nunc, Gaithersburg, MD) were coated with 1 μg/ml TT in PBS. Plates were washed with PBS/0.05% Tween 20 and blocked with 1% bovine serum albumin (BSA) (Fisher Scientific, Pittsburgh, PA). Samples were serially diluted in PBS/0.1% BSA/0.05% Tween 20 (PBST) and added to wells in triplicate. Following incubation, plates were washed and goat anti-mouse IgG γ chain specific horseradish peroxidase (HRP) labeled conjugate (Southern Biotechnology Associates Inc., Birmingham, AL) was added in PBST. After further incubation, antibody binding was detected with substrate buffer containing TMB (Sigma-Aldrich). Absorbance was read with an EIA reader (Molecular Devices, Sunnyvale, CA). Antibody titer was defined as the reciprocal of the dilution of serum that would yield an optical density of 0.5.

Serum samples were collected at weeks 2 and 5, and analyzed for IgG anti-TT antibodies by ELISA. The numerical IgG antibody titer data taken two weeks and five weeks following administration is presented in Exhibit C. At five weeks after a single injection, the response in animals receiving TT/F127/chitosan was significantly higher than that to TT in either component alone ($p = 0.02$ vs. TT/chitosan and $p = 0.0006$ vs. TT/F127) with the outlier removed. Figure 13 graphically summarizes IgG antibody titer data for tests on compositions (i), (iv) and (vii).

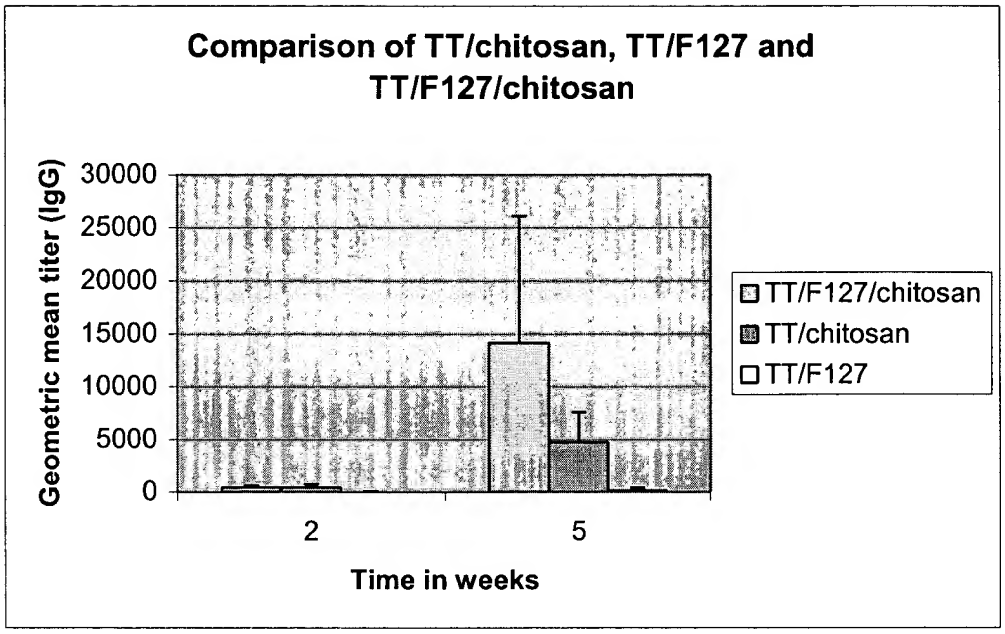

FIGURE 13

EXAMPLE 13: TT with CpG-containing adjuvant in the composition and comparison to other delivery vehicles

Preparation of formulations:. TT and Pluronic® F127 stock solutions were prepared as described in Example 1. ImmunEasy™ containing CpG as an adjuvant was added to formulations in an amount to provide a dose of 20 µl of the ImmunEasy™ per mouse. Tetanus toxoid (TT; Accurate Chemical & Scientific, Westbury, NY) contained 1058 Lf/ml and 2204 Lf/mg protein nitrogen. The various stock solutions were mixed together to form vaccine compositions for testing, as follows:

(i) TT (5 Lf/ml), 20% (v/w) ImmunEasy™ and 16.25% (w/w) Pluronic® F127;
(ii) TT (5 Lf/ml) and 20% (v/w) ImmunEasy™ (no Pluronic® F127);
(iii) TT (5 Lf/ml), 20% (v/w) ImmunEasy™ formulated with glycerol (no Pluronic® F127); and
(iv) TT (5 Lf/ml) and 20% (v/w) ImmunEasy™ formulated with incomplete Freund's adjuvant (no Pluronic® F127).

For composition (iii) TT/ImmunEasy™ in glycerol was prepared by mixing glycerol (approximately 99%; Sigma-Aldrich) with premixed TT/ImmunEasy™ in PBS. For composition (iv), TT in incomplete Freund's adjuvant (IFA) was prepared by emulsification of equal volumes of IFA (Sigma-Aldrich) and a 2x TT/ ImmunEasy™ mixture in PBS.

Immunization studies in mice: Balb/c female mice (Harlan, Indianapolis, IN), 6 to 8 weeks of age, were used for these studies. Groups of mice (n=4) were immunized once s.c. with 0.5 Lf TT in the various formulations on day 0.

Antibody assays: The serum antibody responses to TT were measured by ELISA. Wells of 96 well Nunc Maxisorb microtiter plates (Nunc, Gaithersburg, MD) were coated with 1 μg/ml TT in PBS. Plates were washed with PBS/0.05% Tween 20 and blocked with 1% bovine serum albumin (BSA) (Fisher Scientific, Pittsburgh, PA). Samples were serially diluted in PBS/0.1% BSA/0.05% Tween 20 (PBST) and added to wells in triplicate. Following incubation, plates were washed and goat anti-mouse IgG γ chain specific horseradish peroxidase (HRP)-labeled conjugate (Southern Biotechnology Associates Inc., Birmingham, AL) was added in PBST. After further incubation, antibody binding was detected with substrate buffer containing TMB (Sigma-Aldrich). Absorbance was read with an EIA reader (Molecular Devices, Sunnyvale, CA). Antibody titer was defined as the reciprocal of the dilution of serum that would yield an optical density of 0.5.

Serum samples were periodically collected over a 28 week period and analyzed for IgG anti-TT antibodies by ELISA. The numerical IgG antibody titer data at various time points following administration is presented in Exhibit D. Figure 14 graphically summarizes the IgG antibody titer data through week 16. Data from a representative experiment indicate that at 4 and 8 weeks, the presence of the Pluronic® F127 polymer significantly enhanced the IgG antibody response to TT compared to antigen/ImmunEasy™ alone ($p = 0.0023$ and 0.029 respectively). Furthermore, the response to TT/F127/ImmunEasy™ was significantly higher than that elicited by TT/ImmunEasy™/IFA ($p = 0.017$ and 0.029 respectively). TT/ImmunEasy™ was also combined with glycerol to make a comparison with another matrix used as both a cryoprotectant and a sustained release vehicle. However, this formulation caused no increase in the anti-TT immune response compared to TT/ImmunEasy™ .

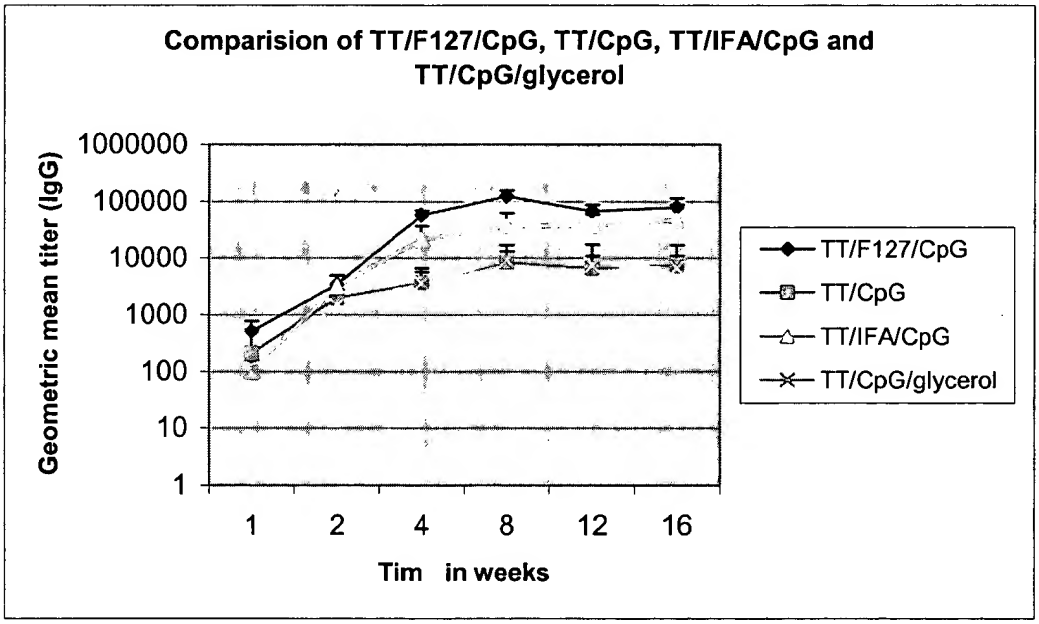

FIGURE 14

EXAMPLE 14: TT with CpG-containing adjuvant in the composition

Preparation of formulations: TT and Pluronic® F127 stock solutions were prepared as described in Example 1. ImmunEasy™ containing CpG as an adjuvant was added to formulations in an amount to provide a dose of 20μl, 6.7 μl or 2 μl of the ImmunEasy™ per mouse. Tetanus toxoid (TT; Accurate Chemical & Scientific, Westbury, NY) contained 1058 Lf/ml and 2204 Lf/mg protein nitrogen. The various stock solutions were mixed together to form vaccine compositions for testing, as follows:

(i) TT (5Lf/ml), 20% (v/w) ImmunEasy™ and 16.25% (w/w) Pluronic® F127;
(ii) TT (5 Lf/ml), 6.7% (v/w) ImmunEasy™ and 16.25% (w/w) Pluronic® F127;
(iii) TT (5 Lf/ml), 2% (v/w) ImmunEasy™ and 16.25% (w/w) Pluronic® F127;
(iv) TT (5 Lf/ml) and 20% (v/w) ImmunEasy™ (no Pluronic® F127);
(v) TT (5 Lf/ml) and 6.7% (v/w) ImmunEasy™ (no Pluronic® F127);
(vi) TT (5 Lf/ml) and 2% (v/w) ImmunEasy™ (no Pluronic® F127; and
(vii) TT (5 Lf/ml) and 16.25% (w/w) Pluronic® F127 (no ImmunEasy™).

Immunization studies in mice: Balb/c female mice (Harlan, Indianapolis, IN), 6 to 8 weeks of age, were used for these studies. Groups of mice (n=8) were immunized once s.c. with 0.5 Lf TT in the various formulations on day 0.

Antibody assays: The serum antibody responses to TT were measured by ELISA. Wells of 96 well Nunc Maxisorb microtiter plates (Nunc, Gaithersburg, MD) were coated with 1 μg/ml TT in PBS. Plates were washed with PBS/0.05% Tween 20 and blocked with 1% bovine serum albumin (BSA) (Fisher Scientific, Pittsburgh, PA). Samples were serially diluted in PBS/0.1% BSA/0.05% Tween 20 (PBST) and added to wells in triplicate. Following incubation, plates were washed and goat anti-mouse IgG γ chain specific horseradish peroxidase (HRP) labeled conjugate (Southern Biotechnology Associates Inc., Birmingham, AL) was added in PBST. After further incubation, antibody binding was detected with substrate buffer containing TMB (Sigma-Aldrich). Absorbance was read with an EIA reader (Molecular Devices, Sunnyvale, CA). Antibody titer was defined as the reciprocal of the dilution of serum that would yield an optical density of 0.5.

Serum samples were collected at weeks 2, 4, and 8 and assayed for the presence of IgG anti-TT antibodies by ELISA. The numerical IgG antibody titer data at various time points following administration is presented in Exhibit E. Figure 15 graphically summarizes IgG antibody titer data for compositions (iii), (vi) and (vii). The data indicate, for example, that at week 4, the formulation of TT with F127/ImmunEasy™(2%) already elicits a significantly higher response than that elicited by either component mixed with antigen alone ($p = 0.001$ vs. TT/ImmunEasy™ and $p = 0.0003$ vs. TT/F127).

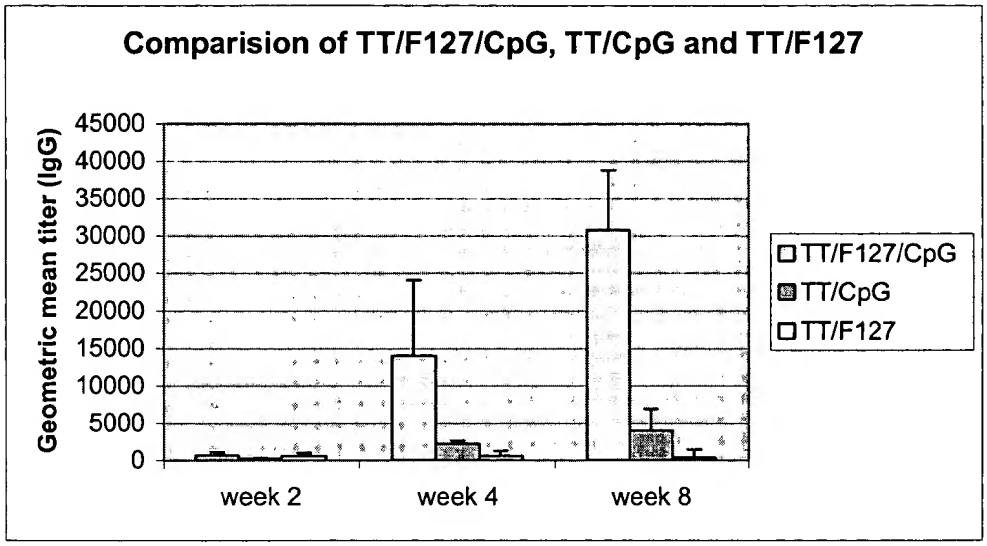

FIGURE 15

EXAMPLE 15: DT with CpG-containing adjuvant in the composition

Preparation of formulations: ImmunEasy™ containing CpG as an adjuvant was added to formulations in an amount to provide a dose of 20 µl of the ImmunEasy™ per mouse. Diphtheria toxoid (DT; Accurate) contained 2100 Lf/ml and 1667 Lf/mg protein nitrogen. The various stock solutions were mixed together to form vaccine compositions for testing, as follows:

(i) DT (1 Lf/dose), 20% (v/w) ImmunEasy™ and 16.25% (w/w) Pluronic® F127 and
(ii) DT (1 Lf/dose) and 20% (v/w) ImmunEasy™ (no Pluronic® F127).

Immunization studies in mice: Balb/c female mice (Harlan), 6 to 8 weeks of age, were used for these studies. Groups of mice (n=4) were immunized subcutaneously (s.c) with 1 Lf DT in the various formulations on day 0.

Antibody assays: The serum antibody responses to DT were measured by ELISA. Wells of 96 well Nunc Maxisorb microtiter plates (Nunc, Gaithersburg, MD) were coated with 10µg/ml DT in PBS. Plates were washed with PBS/0.05% Tween 20 and blocked with 1% bovine serum albumin (BSA) (Fisher Scientific, Pittsburgh, PA). Samples were serially diluted in PBS/0.1% BSA/0.05% Tween 20 (PBST) and added to wells in triplicate. Following incubation, plates were washed and goat anti-mouse IgG γ chain specific horseradish peroxidase (HRP)-labeled conjugate (Southern Biotechnology Associates Inc., Birmingham, AL) was added in PBST. After further incubation, antibody binding was detected with substrate buffer containing TMB (Sigma-Aldrich). Absorbance was read with an EIA reader (Molecular Devices, Sunnyvale, CA). Antibody titer was defined as the reciprocal of the dilution of serum that would yield an optical density of 0.5.

Serum samples were periodically collected over a 32 week period and analyzed for IgG anti-TT antibodies by ELISA. The numerical IgG antibody titer data at various time points following

administration is presented in Exhibit F. Figure 16 graphically summarizes IgG antibody titer data. Data from this experiment indicate, for example, that at 4 and 8 weeks after a single injection, the presence of the Pluronic® F127 polymer and ImmunEasy™ (composition (i)) antigen enhanced the IgG antibody response to DT compared to the use of ImmunEasy™ alone (composition (ii)).

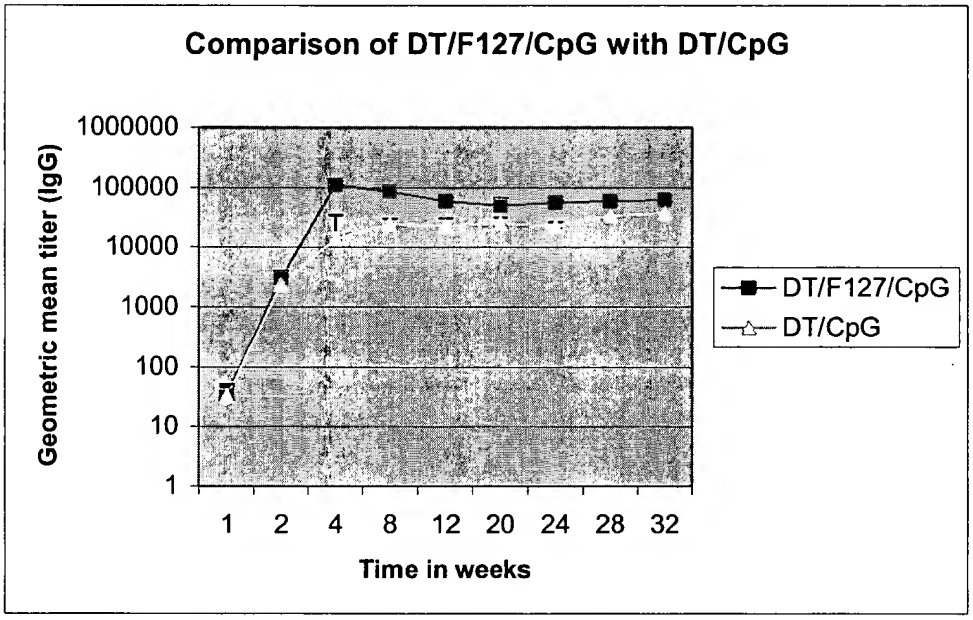

FIGURE 16

EXAMPLE 16: rPA with CpG-containing adjuvant in the composition

Preparation of formulations: ImmunEasy™ containing CpG as an adjuvant was added to formulations in an amount to provide a dose of 20 µl of the ImmunEasy™ per mouse. rPA was obtained from the NIH in the form of a lyophilized protein in 5 mM Hepes, pH 7.4. It was reconstituted in sterile water (USP grade) at 2 mg/ml before formulation. The various stock solutions were mixed together to form vaccine compositions for testing, as follows:

(i) rPA (250µg/ml), 20% (v/w) ImmunEasy™ and 16.25% (w/w) Pluronic® F127 and
(ii) rPA (250µg/ml) and 20% (v/w) ImmunEasy™ (no Pluronic® F127).

Also prepared was a third vaccine composition for testing, as follows:

(iii) rPA adsorbed to aluminum hydroxide (alum) was prepared by adsorption of rPA to Imject® alum (Pierce Endogen, Rockford, IL) according to manufacturer's instructions.

Immunization studies in mice: Balb/c female mice (Harlan, Indianapolis, IN), 6 to 8 weeks of age, were used for these studies. Groups of mice (n=6) were immunized s.c with 25µg rPA in the various formulations on day 0.

Antibody assays: The serum antibody response to rPA was measured by ELISA. The protective capacity of antibodies was measured in vitro using a toxin neutralization assay. For ELISA, wells of 96 well Nunc Maxisorb microtiter plates (Nunc, Gaithersburg, MD) were coated with 1 μg/ml rPA in PBS. Plates were washed with PBS/0.05% Tween 20 and blocked with 1% bovine serum albumin (BSA) (Fisher Scientific, Pittsburgh, PA). Samples were serially diluted in PBS/0.1% BSA/0.05% Tween 20 (PBST) and added to wells in triplicate. Following incubation, plates were washed and goat anti-mouse IgG γ chain specific horseradish peroxidase (HRP)-labeled conjugate (Southern Biotechnology Associates Inc., Birmingham, AL) was added in PBST. After further incubation, antibody binding was detected with substrate buffer containing TMB (Sigma-Aldrich). Absorbance was read with an EIA reader (Molecular Devices, Sunnyvale, CA). Antibody titer was defined as the reciprocal of the dilution of serum that would yield an optical density of 0.5.

Serum samples were periodically collected over an 12 week period and analyzed for IgG antibodies by ELISA. Figure 17 graphically summarizes IgG antibody titer data. The data indicate that rPA/F127/ ImmunEasy™ induced an early rise in IgG antibodies and that this response was significantly higher than the response to rPA/alum ($p < 0.05$).

Toxin Neutralization Assay (TNA): Serum samples were tested for their ability to prevent the lethal toxin (protective antigen + lethal factor (LF))-induced mortality of J774A.1 cells (American Type Culture Collection, Manassas, VA). Recombinant LF (rLF) was obtained from the NIH. Aliquots of 0.2 ml cell suspension (6 to $8x10^5$ cells/ml) in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) were plated into flat 96-well cell culture plates (Corning Costar, Acton, MA). Serial dilutions of pre- and post-immune serum samples were made in TSTA buffer (50 mM Tris pH 7.6, 142 mM sodium chloride, 0.05% sodium azide, 0.05% Tween 20, 2% BSA). PA and LF at final concentrations of 50 and 40 ng/ml respectively were added to each antiserum dilution. After incubation for 1 hour, 10 μl of each of the antiserum-toxin complex mixtures was added to 100 μl of J774A.1 cell suspension. The plates were incubated for 5 hours at 37°C in 5% CO_2. Twenty-five μl of 3-[4,5-dimethyl-thiazol-2-y-]-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) at 5 mg/ml in PBS was then added per well. After 2 hour incubation, cells were lysed and the reduced purple formazan solubilized by adding 20% (w/v) sodium dodecyl sulfate (SDS) in 50% dimethylformamide, pH 4.7. OD was read at 570 nm on an EIA reader. The lethal toxin-neutralizing antibody titers of individual serum samples, calculated by linear regression analysis, were expressed as the reciprocal of the antibody dilution preventing 50% of cell death and normalized to a control rabbit anti-rPA hyperimmune serum (NIH). Pre and post-immunization serum toxin neutralization titers were compared by the Sign test. Toxin neutralization titers between groups were compared by the use of the Mann Whitney U test. P values less than or equal to 0.05 were considered to indicate a significant difference.

The functional nature of the immune response to rPA was measured by TNA. The results of these studies (summarized graphically in Figure 18) indicate that formulation of rPA with F127/ ImmunEasy™ induces toxin neutralization titers significantly higher than formulation of rPA with alum ($p=0.002$) and rPA with ImmunEasy™ ($p=0.041$). The TNA titers were measured 8 weeks post immunization.

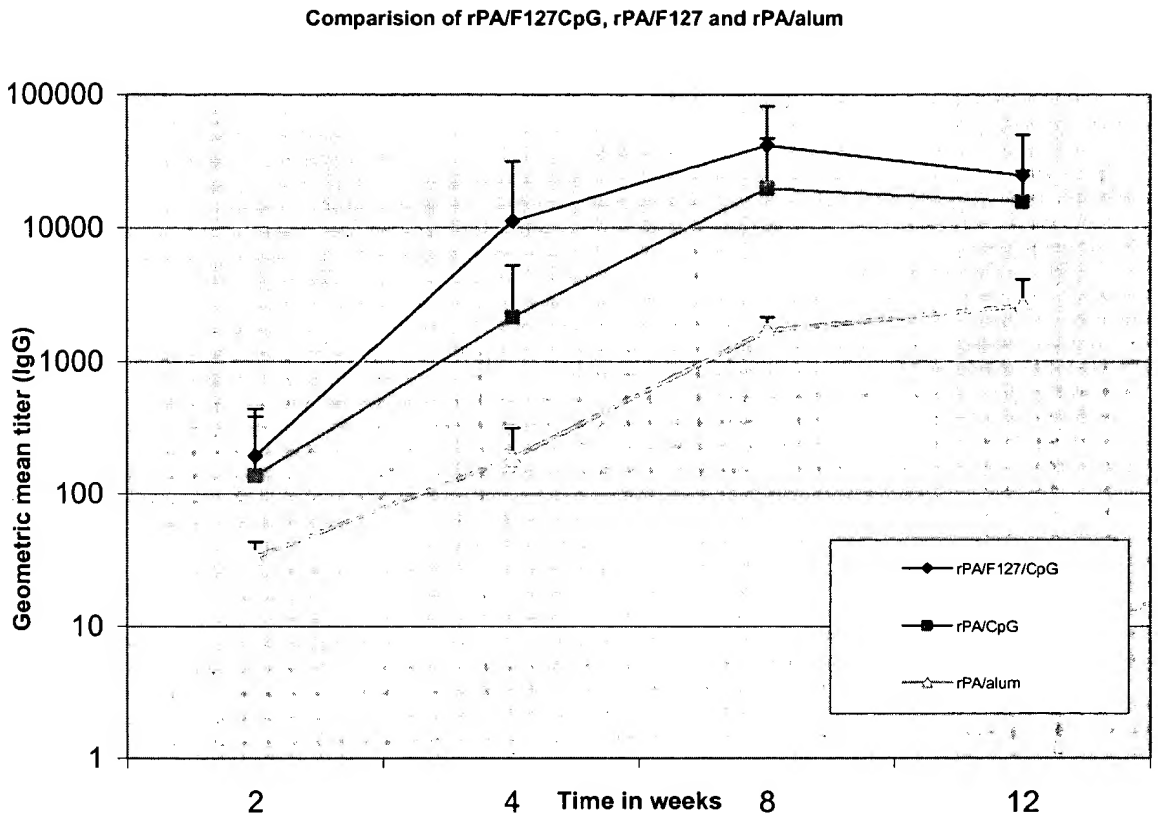

FIGURE 17

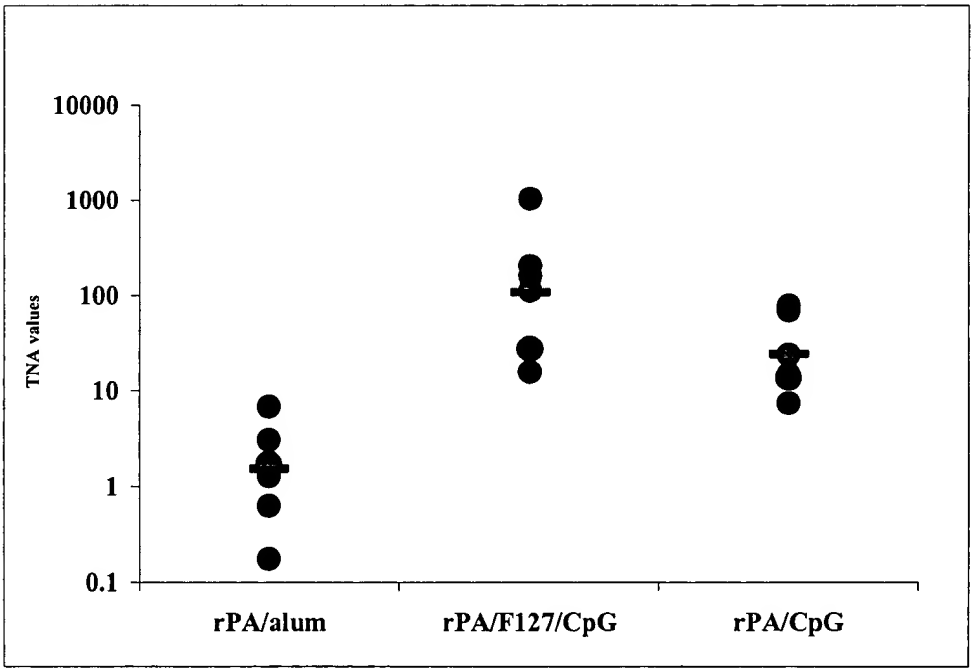

FIGURE 18

EXHIBIT C
TO RULE 132 DECLARATION OF
CLAIRE M. COESHOTT

EXAMPLE 12 – IgG ANTIBODY TITER DATA – CHITOSAN ADJUVANT

Formulation	**Animal**	**2 week**	**5 week**
0.5Lf TT/F127/Protasan® 0.5%	mouse 1-0	509	13187
	mouse 1-1	741	37839
	mouse 1-2	384	23253
	mouse 1-3	198	5989
	mouse 1-4	356	23257
	mouse 1-5	647	29727
	mouse 1-6	298	15262
	mouse 1-7	429	2170
	Geomean	**413**	**14132**
	Average	**445**	**18836**
	StDev	180	11990
0.5Lf TT/F127/Protasan® 0.17%	mouse 2-0	756	24180
	mouse 2-1	452	17380
	mouse 2-2	481	14339
	mouse 2-3	213	12748
	mouse 2-4	510	8873
	mouse 2-5	493	7991
	mouse 2-6	190	2610
	mouse 2-7	2227	17432
	Geomean	**497**	**11201**
	Average	**665**	**13194**
	StDev	656	6713
0.5Lf TT/F127/Protasan® 0.05%	mouse 3-0	279	2350
	mouse 3-1	184	3623
	mouse 3-2	357	3969
	mouse 3-3	359	6209
	mouse 3-4	92	5001
	mouse 3-5	298	7845
	mouse 3-6	290	23496
	mouse 3-7	310	3949
	Geomean	**252**	**5437**
	Average	**271**	**7055**
	StDev	91	6855

0.5Lf TT/Protasan® 0.5%	mouse 4-0	881	*29039
	mouse 4-1	291	5798
	mouse 4-2	13	4031
	mouse 4-3	698	9871
	mouse 4-4	8	3830
	mouse 4-5	DECEASED	DECEASED
	mouse 4-6	624	7050
	mouse 4-7	256	1840
	Geomean	**162**	**4748**
	Average	**396**	**5403**
	StDev	343	2824
0.5Lf TT/Protasan® 0.17%	mouse 5-0	171	4481
	mouse 5-1	620	8617
	mouse 5-2	409	11043
	mouse 5-3	1382	29896
	mouse 5-4	291	10183
	mouse 5-5	DECEASED	DECEASED
	mouse 5-6	299	DECEASED
	mouse 5-7	95	4431
	Geomean	**337**	**9119**
	Average	**467**	**11442**
	StDev	438	9465
0.5Lf TT/Protasan® 0.05%	mouse 6-0	377	14878
	mouse 6-1	292	9444
	mouse 6-2	179	6055
	mouse 6-3	345	8360
	mouse 6-4	144	3782
	mouse 6-5	176	2365
	mouse 6-6	273	2117
	mouse 6-7	98	2317
	Geomean	**215**	**4862**
	Average	**236**	**6165**
	StDev	100	4517
0.5Lf TT/F127	mouse 7-0	2	2
	mouse 7-1	25	193
	mouse 7-2	68	540
	mouse 7-3	85	252
	mouse 7-4	3	11
	mouse 7-5	163	431
	mouse 7-6	71	407
	mouse 7-7	31	472
	Geomean	**27**	**122**
	Average	**56**	**289**
	StDev	53	207

* outlier by Grubb's test not included in the analysis

EXHIBIT D

TO RULE 132 DECLARATION OF CLAIRE M. COESHOTT

EXAMPLE 13-IgG ANTIBODY TITER DATA-CpG ADJUVANT

Formulation	**Animal**	**1 week**	**2 week**	**4 week**	**8 week**	**12 week**	**16 week**	**20 week**	**24 week**	**28 week**
0.5Lf TT/F127/ImmunEasy™	mouse 1-0	922	6126	64626	84766	71894	76790	110056	113677	83564
	mouse 1-1	360	2762	71387	131447	42223	40959	114330	119421	127439
	mouse 1-2	345	2562	49756	129251	75097	102047	117018	109813	95376
	mouse 1-3	567	2936	46479	157242	87592	117944	33722	75090	69229
	Geomean	**505**	**3359**	**57152**	**122672**	**66857**	**78439**	**83943**	**102860**	**91572**
	Average	**549**	**3597**	**58062**	**125677**	**69202**	**84435**	**93782**	**104500**	**93902**
	StDev	269	1693	11886	30089	19219	33574	40142	20000	24783
0.5Lf TT/ImmunEasy™	mouse 2-0	50	1230	1762	6365	4520	2646	5554	7029	7165
	mouse 2-1	800	3118	6679	15961	11966	24294	36885	32372	31118
	mouse 2-2	384	1778	3553	5772	3251	4343	18529	32234	33193
	mouse 2-3	126	2233	4337	8919	10607	10262	22403	22312	16906
	Geomean	**210**	**1975**	**3670**	**8504**	**6572**	**7316**	**17077**	**20113**	**18807**
	Average	**340**	**2090**	**4083**	**9254**	**7586**	**10386**	**20843**	**23487**	**22096**
	StDev	338	799	2039	4675	4340	9830	12896	11940	12307
0.5LfTT/Glycerol/ImmunEasy™	mouse 3-0	118	1147	5779	10536	10957	10054	19997	22691	DECEASED
	mouse 3-1	355	1230	934	3138	1003	1745	2145	1432	DECEASED
	mouse 3-2	97	3602	7924	22344	25604	10425	38030	33281	DECEASED
	mouse 3-3	41	879	3993	9092	10536	10754	17639	18127	DECEASED
	Geomean	**114**	**1451**	**3615**	**9053**	**7379**	**6660**	**13024**	**11833**	
	Average	**153**	**1715**	**4658**	**11278**	**12025**	**8245**	**19453**	**18883**	
	StDev	139	1267	2957	8042	10153	4342	14700	13253	
0.5Lf TT/IFA/ImmunEasy™	mouse 4-0	124	3183	24224	34567	45628	46673	100221	104943	73511
	mouse 4-1	138	5819	29907	48098	51500	63457	83854	123394	126116
	mouse 4-2	42	1830	6884	15554	13082	14419	19158	20535	21779
	mouse 4-3	159	2969	43521	74077	64470	112450	179950	214709	220781
	Geomean	**103**	**3167**	**21584**	**37203**	**37520**	**46812**	**73366**	**86926**	**81711**
	Average	**116**	**3450**	**26134**	**43074**	**43670**	**59250**	**95796**	**115895**	**110547**
	StDev	51	1687	15174	24605	21859	40890	66125	79653	84942

EXHIBIT E
TO RULE 132 DECLARATION OF
CLAIRE M. COESHOTT

EXAMPLE 14 – IgG ANTIBODY TITER DATA – CpG ADJUVANT

Formulation	**Animal**	**2 week**	**4 week**	**8 week**
0.5Lf TT/F127/ImmunEasy™ 20ul	mouse 1-0	2452	4533	6527
	mouse 1-1	8540	10716	21931
	mouse 1-2	6134	7787	16381
	mouse 1-3	10410	5370	18023
	mouse 1-4	7820	18833	178204
	mouse 1-5	5482	DECEASED	DECEASED
	mouse 1-6	13655	25767	191955
	mouse 1-7	7148	155443	111427
	Geomean	**6974**	**14768**	**39903**
	Average	**7705**	**32636**	**77778**
	StDev	3354	54697	81435
0.5Lf TT/F127/ImmunEasy™ 6.7ul	mouse 2-0	1064	43386	227895
	mouse 2-1	3383	100388	101047
	mouse 2-2	1545	129383	173963
	mouse 2-3	2524	107859	146576
	mouse 2-4	1343	13211	658
	mouse 2-5	2197	100320	212735
	mouse 2-6	2930	254421	483347
	mouse 2-7	762	64369	30440
	Geomean	**1761**	**77632**	**76792**
	Average	**1969**	**101667**	**172083**
	StDev	936	72463	149667
0.5Lf TT/F127/ImmunEasy™ 2ul	mouse 3-0	512	19241	42222
	mouse 3-1	201	2002	*273
	mouse 3-2	701	27112	24730
	mouse 3-3	933	27112	23548
	mouse 3-4	708	29535	23802
	mouse 3-5	662	11502	31447
	mouse 3-6	1366	7779	40780
	mouse 3-7	1254	20145	35107
	Geomean	**694**	**14037**	**17065**
	Average	**792**	**18054**	**27739**
	StDev	382	10056	13333

* considered a non-responder removed from plotted data

Formulation	Animal	2 week	4 week	8 week
0.5Lf TT/ImmunEasy™ 20ul	mouse 4-0	3618	2081	2355
	mouse 4-1	2621	1556	2188
	mouse 4-2	5112	7978	12315
	mouse 4-3	10325	20929	131509
	mouse 4-4	5004	5947	1266
	mouse 4-5	9023	7291	19788
	mouse 4-6	4204	15859	658836
	mouse 4-7	6426	4827	136475
	Geomean	**5287**	**6050**	**14429**
	Average	**5792**	**8309**	**46467**
	StDev	2667	6756	57986
0.5Lf TT/ImmunEasy™ 6.7ul	mouse 5-0	523	3678	11148
	mouse 5-1	271	1952	2829
	mouse 5-2	170	2252	6186
	mouse 5-3	368	4555	19027
	mouse 5-4	1082	2005	1479
	mouse 5-5	674	5909	18458
	mouse 5-6	886	3116	17159
	mouse 5-7	458	4309	16667
	Geomean	**476**	**3225**	**8566**
	Average	**554**	**3472**	**11619**
	StDev	311	1411	7247
0.5Lf TT/ImmunEasy™ 2ul	mouse 6-0	254	2217	3005
	mouse 6-1	216	2440	4822
	mouse 6-2	248	1870	2360
	mouse 6-3	253	2122	6307
	mouse 6-4	373	3133	6657
	mouse 6-5	361	1757	2545
	mouse 6-6	456	1978	1897
	mouse 6-7	113	2741	10116
	Geomean	**264**	**2243**	**4034**
	Average	**284**	**2282**	**4714**
	StDev	107	467	2844

0.5Lf TT/F127	mouse 7-0	402	375	553
	mouse 7-1	880	763	965
	mouse 7-2	1025	727	273
	mouse 7-3	100	64	2
	mouse 7-4	1074	1548	1347
	mouse 7-5	1069	851	222
	mouse 7-6	1261	2104	*3364
	mouse 7-7	431	641	682
	Geomean	**623**	**626**	**345**
	Average	**780**	**884**	**926**
	StDev	414	650	1076

* outlier by Grubb's

EXHIBIT F
TO RULE 132 DECLARATION OF
CLAIR M. COESHOTT

EXAMPLE 15 – IgG ANTIBODY TITER DATA – CpG ADJUVANT

Formulation	**Animal**	**1 week**	**2 week**	**4 week**	**8 week**	**12 week**	**16 week**	**20 week**	**24 week**	**28 week**	**32 week**
1 Lf DT/ImmunEasy™	mouse 5-0	29	2497	50487	30118	33306	27301	28398	23309	28687	25079
	mouse 5-1	42	3391	6652	22451	24894	25266	26298	9622	32729	49216
	mouse 5-2	58	1325	14953	16828	14477	15250	18644	19928	20736	22238
	mouse 5-3	14	2567	7072	27854	20336	27631	28410	48629	66663	70600
	Geomean	**32**	**2317**	**13728**	**23727**	**22227**	**23219**	**25079**	**21592**	**33753**	**37310**
	Average	**36**	**2445**	**19791**	**24313**	**23253**	**23862**	**25438**	**25372**	**37204**	**41783**
	StDev	19	850	20817	5937	7943	5836	4637	16561	20262	22706
1 Lf DT/F127/ImmunEasy™	mouse 6-0	*9	1540	14847	6070	2928	2470	2880	3532	2250	2161
	mouse 6-1	19	2950	105873	69766	49986	37955	46485	49058	49500	47881
	mouse 6-2	37	2161	129855	98378	64515	54542	55037	57870	57101	59940
	mouse 6-3	44	4051	96437	87278	63688	18668	47777	60742	72827	76451
	Geomean	**23**	**2511**	**66609**	**43668**	**27847**	**17577**	**24358**	**27936**	**26087**	**26241**
	Average	**27**	**2676**	**86753**	**65373**	**45279**	**28409**	**38045**	**42801**	**45420**	**46608**
	StDev	16	1083	49959	41252	29010	22670	23743	26647	30375	31862

*mouse 6-0 omitted from graphic as low responder